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Review Article

Functions of BMP signaling in embryonic stem cell fate determination

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ABSTRACT

Embryonic stem (ES) cells, which can self-renew and can differentiate to various cell types, have great potential applications in regenerative medicine. BMP family members are evolutionarily conserved factors that play critical roles in embryogenesis and in adult tissue homeostasis in multicellular organisms, and their malfunction can lead to various human diseases. Consistent with its importance in early embryogenesis, BMP signaling has been established as a key determinant that directs a wide range of cell fate choices in both mouse and human embryonic stem cells, from self-renewal maintenance to multiple differentiation processes. Remarkably, BMPs exert their diverse functions via integrating with signal inputs from other extrinsic signals and intrinsic factors, including transcription factors and epigenetic regulators. Here we summarize the current understanding of BMP signaling in embryonic stem cell fate determination, and discuss the delicate cooperation between BMP signaling and its partners in these processes. The principles learned from these studies would pave a road for the potential medical applications of embryonic stem cells.

Contents

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Introduction

Cells from the inner cell mass (ICM) of early embryos are capable of differentiating to all adult cell lineages and germ cells (pluripotency). However, the pluripotent state in these cells is transient, with gradually narrower developmental potential along the embryo development process. *In vitro* culture of these cells in appropriate conditions successfully established mouse and human embryonic stem (ES) cells, and importantly, these ES cells can be propagated indefinitely while maintaining their pluripotent state [1–3]. The ability to self-renew and differentiate renders ES cells great promise in regenerative medicine, and ES cells have been widely used in disease modeling and drug discovery. To fully exploit the potentials of ES cells in medical application, it is a prerequisite to understand how ES cell fates are determined, i.e. how ES cell self-renewal is maintained and how ES cells can be differentiated to specified cell lineages. Current understanding of ES cells has indicated the complicated and delicate control of ES cell fates at various levels, including extrinsic signals, intrinsic transcription factors and epigenetic regulators [4–8].

Of these extrinsic signals, bone morphogenetic proteins (BMPs) play critical roles in directing both self-renewal and differentiation of ES cells. They are members of the transforming growth factor β (TGF-β) family, and have evolutionarily conserved functions in a wide range of physiological and pathological processes, including embryogenesis, adult tissue homeostasis and various kinds of diseases [9,10]. BMP signaling is initiated when dimeric extracellular BMPs – the ligands – bind to the transmembrane type I and type II receptors, leading to activation of the serine/threonine kinase in the intracellular domain of the type I receptors. Then, the receptor-activated Smads (R-Smads, Smad1/5/8) – the mediators of BMP signaling – are phosphorylated and activated by the type I receptor kinases, resulting in formation of a complex with common Smad (co-Smad, Smad4). The Smad complex is subsequently translocated from the cytoplasm to the nucleus, where they elicit transcriptional responses [10–14]. Consistent with the importance of BMP signal in early embryogenesis, it plays critical roles in a broad spectrum of cell fate choices in ES cells, including the maintenance of self-renewal and specification to all kinds of cell lineages. The cell fate determination is delicately controlled, and increasing evidence suggests that BMP signaling cooperates intimately with other extrinsic signals, intrinsic transcription factors and epigenetic regulators to fully exert its functions in a context-dependent manner. Here we summarize the roles of BMP signaling in guiding ES cell fates, and discuss the crosstalk between BMP signaling and other cell fate regulators.

BMP signaling in ES cell fate determination

BMP signaling in ES cell self-renewal

BMP signaling maintains mouse ES cells in the self-renewal state in cooperation with leukemia inhibitory factor (LIF) signaling [15,16]. Mouse ES cells are routinely cultured on mouse embryonic fibroblast (MEF)-derived feeder cells with LIF cytokines as a supplement. The factors that serum and feeder cells provide to support self-renewal were unclear until in 2003 when Austin Smith's group reported that in a chemically defined culture medium, the addition of BMP4 can bypass the need for both serum and feeder cells [15]. In the BMP plus LIF culture condition, the long-term self-renewal ability can be maintained in mouse ES cells, and withdrawal of either BMP or LIF drives quick and dramatic differentiation of the ES cells. Thus, BMP contributes to self-renewal maintenance in cooperation with LIF.

Several downstream target genes of BMP signaling that contribute to self-renewal maintenance were identified thereafter. Inhibitor of differentiation (*Id*) family proteins are the classic BMP target genes in various cell types, including mouse ES cells [15–17], and thus their contributions to ES cell self-renewal were discovered at the very beginning. IDs suppress precociously expressed neurogenic basic helix-loop-helix (bHLH) transcriptional activators and therein suppress neural differentiation, leading to strengthened self-renewal [15]. Genome-wide analysis further identified dozens of candidate BMP target genes in mouse ES cells, as evidenced by Smad binding in their promoter regions and also regulation of their expression by BMP [18]. Most of these genes are developmental regulators, and their promoter regions are largely marked by both H3K4 and H3K27 trimethylation. The "bivalent marks" keep these genes in a so called "poised state", in which gene expression is suppressed but can be turned on quickly in appropriate conditions [19]. In this scenario, BMP signaling suppresses the expression of a large set of developmental regulators and sustains self-renewal. A key BMP target gene, dual-specificity phosphatase 9 (*Dusp*9) was identified recently, which mediates BMP signaling to suppress intrinsic ERK activity and contributes to robust mouse ES cell self-renewal [16,20]. Taken together, current evidence supports the idea that in mouse ES cells, BMP signaling sustains self-renewal via regulation of a cohort of downstream target genes (Fig. 1).

However, in human ES cells, it is a quite different situation, where BMP is a strong signal to induce differentiation and maintaining low BMP signal is necessary for human ES cells to keep self-renewal. In feeder-free conditions, human ES cells are cultured in medium that contains knockout serum replacement (KSR) supplemented with basic fibroblast growth factor (bFGF, or FGF2) [21]. This medium is conditioned on MEF derived feeder cells before feeding ES cells (conditioned medium, CM), and if the medium is unconditioned, i.e. only KSR and bFGF, but without factors from the feeder cells (unconditioned medium, UM), human ES cells will differentiate. It was observed that in UM, phosphorylation of Smad1/5/8 was much higher than that in CM [22,23], and inhibition of BMP signaling with its antagonist Noggin reduced Smad1/5/8 phosphorylation and at the same time, maintained long-term self-renewal of human ES cells in UM [23,24]. The apparently opposite roles that BMP plays in mouse and human ES cells may be attributed to the developmental stages that mouse and human ES cells represent. Human ES cells are regarded as the counterpart for the more elongated epiblast stage cells while mouse ES cells are believed to resemble the inner cell mass in the blastocyst stage, which is considered as more primitive, or so called "naive" state [25]. In contrast, similar functions of BMP signaling have been reported in both human and mouse ES cells in the context of differentiation (see below).